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RESEARCH PAPER

Piroxicam Encapsulated in Liposomes: Characterization and In Vivo Evaluation of Topical Anti-inflammatory Effect

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ABSTRACT

Liposomes of soya phosphatidylcholine, cholesterol, and stearylamine (molar ratio 6/3/1) and 0.1% α-tocopherol were prepared by the extrusion of multilamellar vesicles through 0.2-μm polycarbonate membrane. Liposomes were characterized by electron transmission microscopy, and the mean structure diameter was 278 nm. The encapsulation efficiency obtained was 12.73%. The topical anti-inflammatory effect was evaluated in vivo by the cotton pellet granuloma method. We analyzed free piroxicam at 4 mg/kg, piroxicam encapsulated in liposomes added to 1.5% hydroxyethylcellulose (HEC) gel at 1.6 mg/kg, and piroxicam encapsulated in liposomes added to HEC gel at 4 mg/kg; the inhibition of inflammation obtained was 21.1%, 32.8%, and 47.4%, respectively. These results showed that the encapsulation of piroxicam produced an increase of topical anti-inflammatory effect, suggesting that the inhibition of inflammation can be obtained with lower drug concentrations.
Key Words: *In vivo evaluation; Liposomes; Piroxicam; Topical application.*

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INTRODUCTION

Liposomes have been extensively studied as a drug delivery system and can improve biological efficacy and reduce side effects of drugs (1). Topical application of liposome preparations has been shown to enable the penetration of entrapped drugs, increasing the local effect and reducing systemic absorption in comparison to the classical release systems (2–5). Liposomes were considered more suitable formulations due to their ability to permit sustained drug release (6). These structures have been investigated for the local treatment of the inflammatory process (7,8).

Piroxicam is a nonsteroidal anti-inflammatory compound and has analgesic, anti-inflammatory, and antipyretic activities. Piroxicam controls the inflammatory process of arthritic disease and other inflammatory conditions. Nevertheless, it has been associated with gastrointestinal disturbances, including ulceration and hemorrhage (9,10). Comparative studies using animal models show that the topical anti-inflammatory effect of free piroxicam was similar to that from oral and rectal administration (10,11). Although it is possible to overcome these problems by planning drug carriers to prevent direct contact of drug with the gastric mucosa or that allow the topical administration of the drug (10,12–16). Since liposomes containing phospholipid are nontoxic and biodegradable vehicles for drug release (17), the purpose of this investigation was to study the preparation and characterization of piroxicam encapsulated in liposomes and the effects of liposomes in the topical anti-inflammatory activity of piroxicam.

MATERIALS AND METHODS

Piroxicam (lot f301) was kindly donated by ANSA (Milano, Italy); other materials were soya phosphatidylcholine (Epikuron 200; Lucas Meyer, Paris, France), cholesterol, tris (hydroxymethyl)aminomethane and ethyl ether (Merck, Darmstadt, F.R. Germany), stearylamine (Sigma, St. Louis, MO), DL-α-tocopherol acetate (Hoffmann-La Roche, Ltd., Basel, Switzerland), Sephadex G-50 medium (Pharmacia Biotech, Uppsala, Sweden), and hydroxyethylcellulose (HEC; Union Carbide). All other solvents and chemicals were analytical grade.

Liposome Preparation

Liposomes were prepared by the ''film'' method (18,19) from a mixture of soya phosphatidylcholine, cholesterol, and stearylamine (molar ratio 6:3:1). The total lipid concentration was 60 mM, and 0.1% of α-tocopherol acetate was added as an antioxidant. The piroxicam concentration was 10 mg/ml. Lipids and piroxicam were dissolved in 5 ml of chloroform into a 250-ml round-bottom flask. Solvent was removed under vacuum in a rotary evaporator at 35°C ± 0.5°C and 60 rpm to obtain a smooth, dry lipid film. The process continued for 15 min after the dry film first appeared. Then, the film was hydrated with Tris-HCl buffer 0.05 mM, pH 7.4, with 0.5 g of glass beads. The flask was kept rotating for 30 min or until a homogeneous milky white suspension was formed. The suspension was allowed to stand an additional 2 hr at room temperature to complete the swelling process. The dispersion was stored in a refrigerator at 4°C under nitrogen atmosphere. Phospholipid was analyzed quantitatively by the ultraviolet-visible (UV-Vis) procedure using Stewart's method (20).

The size of multilamellar vesicles (MLVs) was reduced by extrusion through a 0.2-μm polycarbonate membrane. After 10 cycles of extrusion, the liposome samples remained completely homogeneous.

Free Drug Exclusion

The nonencapsulated drug was separated from the loaded liposomes using the minicolumn centrifugation method (21). A 5-ml disposable plastic syringe was filled with Sephadex G-50 (medium) in saline solution. The columns were centrifuged at 2000 rpm for 3 min at room temperature to remove the saline solution. Then, 1 ml of buffered liposome dispersion was added carefully on top of the Sephadex gel, and the columns were centrifuged at 2000 rpm for 3 min. The elutes from each tube were set aside for quantitative analysis. Subsequently, 0.5 ml of the Tris-HCl buffer 0.05M, pH 7.4, was applied, and the same condition of centrifugation was used. This step was repeated, but 1 ml of buffer was applied until all free material came out of the column.

Determination of the Encapsulated Drug

The piroxicam content was determined quantitatively by UV-VIS spectrophotometry at 330 nm, solubilizing 10 μl of liposome dispersion into 2 ml of absolute ethanol. Empty liposomes with the same composition were dissolved in the same solvent and used as controls.

Drug Encapsulation Efficiency

Efficiency of encapsulation E_c was evaluated by the percentage of the drug encapsulated according to the following expression:

$$E_c = (\text{Total encapsulated drug} \times 100)/\text{Total drug}$$

Determination of the Internal Volume

The volume of encapsulated aqueous phase was determined by the following expression:

$$V_c = (\%\ \text{Encapsulated} \times \text{VDS})/(100 \times \text{Total amphiphile [mg]})$$

where VDS is the volume of drug solution (μl).

Gel Preparation

Gel containing HEC 1.5% (w/w) and methylparaben 0.15% (w/w) was prepared in Tris-HCl buffer 0.05M, pH 7.4, at 50°C. The liposome dispersion was incorporated in the HEC gel at room temperature.

Determination of Liposome Diameter

The diameters of the vesicles were determined by a negative stain electron microscopy method in a transmission microscope (Zeiss model EM10, Oberkochem, Germany) with 1% of phosphotungstic acid (22). The samples used were piroxicam liposome dispersion after extrusion and piroxicam liposomes incorporated in HEC gel. The liposome mean diameter was determined by the analysis of 200 structures from 10 photomicrographs. An exponential sampling method was used for data analysis. The mean diameter was obtained through the plot of the accumulated frequency in probability scale versus the log of diameters.

Determination of the In Vivo Anti-inflammatory Effect

The anti-inflammatory activity of piroxicam was carried out by the cotton pellet granulome method (23,24). Wistar adult rats weighing 150–170 g were separated into six groups of animals. The animals were anesthetized with ethyl ether inhalation, and four cotton cylinders (40 mg each) were implanted in the subcutaneous tissue in four dorsal sites of each animal. Then, HEC gel containing free piroxicam 0.27% (w/w) in a dose of 4 mg/kg (G1) and HEC gel with liposomes of piroxicam 0.13% (w/w) in doses of 4 mg/kg (G2) and 1.6 mg/kg (G3) were applied 2 hr later and every 24 hr for 6 successive days. On day 7, the animals were sacrificed by ethyl ether inhalation, and the granulomes were extracted. The granulomes were dried at 60°C for 24 hr and then immediately weighed in an analytical balance. The results were analyzed by the Student *t* test with a 5% significance level.

Table 1

Parameters of Liposome Characterization

E_c = 12.73%
V_c = 4.49 μl/μml
Liposome mean diameter = 278 nm
Phospholipid content = 28.3 mg/ml

RESULTS

The results of the liposome characterization are shown in Table 1.

The effect of daily topical application of formulations G1, G2, and G3 on the inhibition of the granuloma formation is showed in Table 2 and represented in Fig. 1.

Figure 1 shows the effect of daily topical administration of piroxicam formulations and control on the granulomatous tissue formation.

The in vivo anti-inflammatory effect of free piroxicam incorporated in HEC gel and of piroxicam loaded in liposomes incorporated in HEC gel is showed in Fig. 2.

DISCUSSION

Liposomes can dissolve molecules that are sparingly water soluble and act as microreservoirs with controllable leakage characteristic. The literature shows that several drugs, like antibiotics, analgesics, antifungals, and nonsteroidal anti-inflammatory liposomes, can improve the therapeutic effect (25). The growing use of liposomes as drug delivery systems requires potential alterations to those features of the bilayer that may affect the barrier properties of the skin in order to improve bioavailability and therapeutic index.

Our experimental results confirm the ability of liposomes to alter the therapeutic effect of piroxicam. The encapsulation of piroxicam in liposomes produced an enhancement of the anti-inflammatory effect compared with free drug (Fig. 1). Piroxicam in G2 and G3 formulations in the presence of HEC gel caused inhibition of the inflammation of 47.4% and 32.78%, respectively (Fig. 2). This inhibition by free piroxicam in the same gel in a dose of 4 mg/kg was only 21.09%. The gel did not affect the anti-inflammatory activity of the piroxicam loaded in liposomes, probably because the lipid vesicles, due to its chemical composition and bilayer structure, can

Table 2

Determination of Topical Anti-inflammatory Effect of Formulations G1, G2, and G3

	Dry Granuloma Weight, Day 7 (mg)					
	CT	G1 (4 mg/kg)	CT	G2 (4 mg/kg)	CT	G3 (1.6 mg/kg)
1	355.5	278.0	322.7	185.8	306.4	238.3
2	342.9	250.8	286.8	175.4	295.9	195.9
3	313.6	253.7	390.6	157.2	301.1	189.8
4	341.0	267.4	309.6	142.2	346.0	189.1
5	320.3	253.8	304.0	172.6	269.5	196.6
6	330.6	261.9	290.6	166.6	303.5	215.3
Mean standard	330.6	260.93	317.3	166.64	303.73	204.16
error	4.7	4.24	15.58	6.23	10.05	7.85
Inhibition (%)		21.09		47.4		32.78

CT = control.
$p < .05$ significant in relation to the controls.

interact with the lipidic surface of the skin, which acts as a barrier for hydrophilic drugs (26).

HEC gel is a hydrophilic topical base without properties of skin penetration. Topical absorption of piroxicam will depend, therefore, on its oil/water partition coefficient (27,28). In fact, our results, in the absence of absorption promoter, showed only low skin penetration of piroxicam when incorporated in an epidermal base like the hydrophilic gels.

The enhancement of the anti-inflammatory effect obtained when piroxicam was encapsulated in liposomes probably can be related to the permeation increase due to the amphiphile of the liposome bilayer and to the slow release of the drug from the liposomes. This enables piroxicam to last longer in the locality of its pharmacological effect (6). The drug will be released from the liposomes if the bilayer is destroyed by organic fluids (13). Liposomes can act as a reservoir system, similar to a slow-release vehicle (4, 29,30), enabling more uniform and prolonged release of the drug (17). So, a high topical concentration of the drug can be maintained when compared with conventional vehicles (17,31). The inhibition of the inflammation produced by piroxicam encapsulated in liposomes in the doses of 1.6 mg/kg and 4 mg/kg was 32.78% and 47.4%, respectively. Since, in both cases,

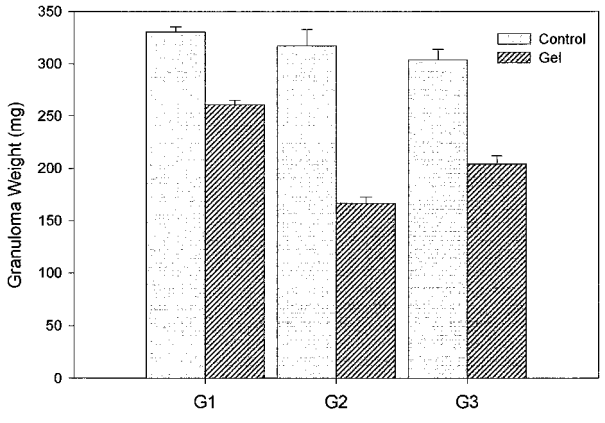

Figure 1. Effect of daily topical application of piroxicam formulations on the granulome weight at day 7. G1 = free piroxicam at 4 mg/kg; G2 = piroxicam liposome in HEC gel at 4 mg/kg; G3 = piroxicam liposome at 1.6 mg/kg.

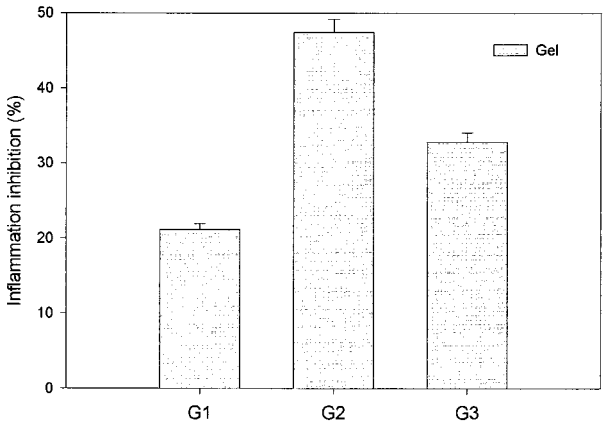

Figure 2. Effect of topical application of piroxicam formulations G1, G2, and G3 on the inhibition of inflammation.

the E_c of piroxicam in liposomes was similar, the higher quantity of liposomes containing piroxicam in the dose of 4 mg/kg may have enabled higher penetration of liposomes into the cutaneous structure. Liposomes may act as percutaneous release vehicles of drugs according to bioavailability studies in vivo, which showed the presence of intact liposomes in the skin (6). In fact, adsorption of phospholipid liposomes on the outer surface of skin and structural changes in deeper layers of the stratum corneum due to mixing of the liposome lipids and the stratum corneum were observed (32).

The liposome encapsulation of piroxicam enabled the reduction of the dose, with increased topical anti-inflammatory activity, compared to the free drug, with a decreased possibility of systemic side effects. Liposomes were shown to be effective as a piroxicam carrier for cutaneous administration.

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REFERENCES

1. D. D. Lasic and D. Papahadjoupoulos, *Medical Application of Liposomes*, Elsevier, New York, 1998.
2. M. Mezei and V. Gulasekharam, Life Sci., 26, 1473–1477 (1980).
3. A. Gursoy, J. Akbuga, and L. J. Eroglu, Pharm. Pharmacol., 40, 53–54 (1987).
4. M. Mezei, Can. Pharm. J., 124, 132–155 (1991).
5. T. Ogiso, N. Niinaka, and M. Iwaki, J. Pharm. Sci., 85, 57–64 (1996).
6. M. Mezei, in *Drug Permeation Enhancement: Theory and Applications* (D. S. Hsieh, Eds.), DNLM, New York, 1994, pp. 171–198.
7. J. Lasch and W. Wohlrab, Biomed. Biochim. Acta, 45, 1295–1299 (1986).
8. H. Benameur, G. Grand, and R. Brasseur, Int. J. Pharm., 89, 157–167 (1993).
9. M. Mihalic', H. Hofman, and J. Kuftinec, *Analytical Profiles Drug Substances*, Academic, San Diego, 1986, pp. 509–531.
10. A. Babar, A. J. C. Solanki, and F. Plakogiannis, Drug Dev. Ind. Pharm., 16, 523–540 (1990).
11. D. L. Larson and J. G. Lombardino, Agents Actions, 10, 246–251 (1980).
12. T. Kageyama, Eur. J. Rheumatol. Inflamm., 8, 114–115 (1987).
13. N. Weiner, F. Martin, and M. Riaz, Drug Dev. Ind. Pharm., 15, 1523–1554 (1989).
14. E. E. Linn, R. C. Pohland, and T. K. Byrd, Drug Dev. Ind. Pharm., 16, 899–920 (1990).
15. N. A. Monteiro-Riviere, A. O. Imman, and J. E. Riviere, Pharm. Res., 10, 1326–1331 (1993).
16. R. Marks and P. Dykes, Skin Pharmacol., 7, 340–344 (1994).
17. D. Lasic, Am. Sci., 80, 20–31 (1992).
18. A. D. Bangham and R. W. Horne, J. Mol. Biol., 8, 660–668 (1964).
19. A. D. Bangham, M. M. Standish, and J. C. Watkins, J. Mol. Biol., 13, 238–252 (1965).
20. R. R. C. New, in *Liposomes: a Practical Approach* (R. R. C. New, Ed.), IRL Press, Oxford, England, 1990, pp. 33–104.
21. D. W. Fry, C. White, and D. Goldman, J. Anal. Biochem., 90, 809 (1978).
22. R. R. C. New, in *Liposomes: a Practical Approach* (R. R. C. New, Ed.), IRL Press, Oxford, England, 1990, pp. 105–161.
23. C. J. E. Niemegeers, W. V. Bruggen, and F. Awouters, Arzneim.-Forsch., 25, 1524–1526 (1975).
24. D. H. P. Germano, T. T. O. Caldeira, and A. A. A. Mazella, Fitoterapia, 54, 459–462 (1993).
25. N. Weiner and L. Lieb, in *Medical Application of Liposomes* (D. D. Lasic and D. Papahadjoupoulos, Eds.), Elsevier, New York, 1998, pp. 493–513.
26. I. R. Fornovi, M. T. G. Rańon, and A. M. Rivera, Afinidad, 46, 460–466 (1989).
27. B. W. Barry, in *Pharmaceutical Dosage Forms: Disperse Systems*, Vol. 1 (H. A. Lieberman, M. M. Rieger, and G. S. Banker, Eds.), DNLM, New York, 1988, pp. 245–283.
28. R. Viglioglia, *Cosmiatria II*, AP Americana, Buenos Aires, 1991, pp. 105–107.
29. E. Touitou, N. Shaco-Ezra, and N. Dayan, J. Pharm. Sci., 81, 131–134 (1992).
30. E. Fattal and F. Puisieux, in *Formes Pharmaceutiques pour application locale* (M. Seiller and M. C. Martini, Eds.), Tec and Doc Lavoisier, Paris, 1996, pp. 396–411.
31. V. Masini, F. Bonte, and A. Meybeck, J. Pharm. Sci., 82, 17–21 (1993).
32. H. E. Hofland, J. A. Bouwstra, H. E. Bodde, F. Spies, and H. E. Junginger, Br. J. Dermatol., 132, 853–866 (1995).